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## DISEASE NOTES



# First Report of Three *Lasiodiplodia* Species (*L. theobromae*, *L. pseudotheobromae*, and *L. caatinguensis*) Causing Cashew Gummosis in Guinea-Bissau (West Africa)

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Since 2013, there have been reports of gummosis on cashew (*Anacardium occidentale* L.) in Guinea-Bissau; however, identification of the causal agent remains to be addressed (Monteiro et al. 2015, 2017). During harvest season in May 2018, cashew trunks with dark cankers, cracks, and resin-like gum were observed in orchards located in Bolama Island of Bijagós archipelago and in the continental Tombali region of Guinea-Bissau. Samples from healthy to diseased margins of trunk lesions were collected, surface disinfected with 7% sodium hypochlorite, washed twice with sterilized water, placed on potato dextrose agar (DIFCO, Sparks, MD) supplemented with potassium thiocyanate (50 µg/ml, Sigma, Algés,

Portugal), and incubated at  $24 \pm 1^\circ\text{C}$ . Fungi with mycelium immersed and superficial, first white and later dark greenish to grayish black, were consistently isolated after 7 days, developing pycnidia with conidia initially hyaline, later becoming one-septate, dark brown with longitudinal striations and thick-walled. These morphological characteristics fit the *Lasiodiplodia* species description (Phillips et al. 2013). Pure cultures from two isolates from Bolama (GB78 and GB134) and one from Tombali (GB32) were used for molecular identification. Genomic DNA was extracted from mycelia using a Qiagen Plant DNA Kit (Qiagen), and partial regions of nuclear ITS rDNA (ITS1F, Gardes and Bruns 1993; ITS4R, White et al. 1990), translation elongation factor 1- $\alpha$  (*TEF1*- $\alpha$ , Alves et al. 2008), and  $\beta$ -tubulin ( *$\beta$ -tub*, Glass and Donaldson 1995) genes were amplified following previously described conditions (Coutinho et al. 2017) modified with the addition of bovine serum albumin (BSA, 50 mg/ml). Sequences generated were deposited in GenBank under the accession numbers MN952990 to MN952992 for *ITS*, MN952201 to MN952203 for  *$\beta$ -tub*, and MN952205 to MN952207 for *TEF1*- $\alpha$ . Phylogenetic analyses from the combined dataset using maximum-likelihood and Bayesian inference methods placed each isolate in well-supported clades, namely GB78 as *L. theobromae*, GB32 as *L. pseudotheobromae*, and GB134 as *L. caatinguensis*. Pathogenicity tests were performed on 3-month-old cashew plants (eight plants per isolate). Inoculation followed the method of Lima et al. (2013), consisting of excising a 3-mm-diameter tissue bark out and replacing it with a 3-mm-diameter PDA plug removed from the colony margin. The inoculation wound was covered with sterilized wet cotton and sealed with parafilm. Plants inoculated only with PDA plugs with the wound covered and sealed as previously described were used as controls. After 15 days, all inoculated plants displayed similar symptoms to those observed in the field, whereas control plants remained symptomless. The pathogens were successfully reisolated from symptomatic stems, fulfilling Koch's postulates. *Lasiodiplodia* spp. have been described as a causal agent of cashew gummosis, an important disease impacting cashew production in northeastern Brazil (Coutinho et al. 2017). Here we report for the first time three *Lasiodiplodia* species (*L. theobromae*, *L. pseudotheobromae*, and *L. caatinguensis*) as causal agents of cashew gummosis in Guinea-Bissau, where this crop is the most important agricultural commodity, and given its high economic value, a potential reduction in production would negatively impact smallholder livelihoods and annual revenues.

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F. Monteiro and I. Diniz contributed equally to this work.

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